Development of a Method To Assess Cigarette Smoke Intake

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Tar and nicotine deliveries of cigarettes measured using current standardized smoking machine protocols provide poor estimates of smoke exposure. The characteristics of human smoking behavior vary considerably and differ from the rigid parameters used with current standardized smoking machine protocols. Current alternatives, including measurement of biomarkers, are invasive, time-dependent, and can be too expensive to be used as mechanisms for carrying out large-scale investigations required to help determine the influence of cigarette design on smoking behaviors. To obtain more reasonable estimates of mainstream smoke exposure, we developed a method to quantitatively measure solanesol, a naturally occurring component in tobacco that is deposited during smoking in the cigarette filter butt. Quantification of solanesol extracted from the filters using liquid chromatography and tandem mass spectrometry is efficient, rapid, and extremely reliable. We found that the amount of solanesol deposited in a cigarette filter is related to the mainstream smoke deliveries of tar and nicotine under a variety of smoking conditions. In addition, the amount of solanesol trapped in the filter remains stable at least 4 weeks after smoking. Measuring solanesol in cigarette filters as an exposure marker provides a noninvasive means to obtain reasonable estimates of mainstream tar and nicotine smoke deliveries under a wide variety of smoking conditions.

Introduction

Standardized machine testing of tar and nicotine in cigarettes resulted from concern that cigarette smoking increased the risk for various diseases including lung cancer (*1*). Because several potent carcinogens had been associated with the tar component of cigarette smoke, the tobacco industry began advertising reduced-tar brands with implied health benefits. As a result the Federal Trade Commission (FTC) became involved with the determination of tar and nicotine yields in mainstream cigarette smoke (*2*). Mainstream smoke is the portion of cigarette smoke that is drawn through the filter butt during a puff. Sidestream smoke refers to the smoke emanating from the smoldering coal tip between puffs. Environmental tobacco smoke (ETS) originates from a combination of sidestream smoke and exhaled mainstream smoke. Several reports concluded that the machine-smoked deliveries of tar, nicotine, and carbon monoxide (CO) in mainstream smoke obtained using the FTC smoking protocol do not reflect a smoker's uptake (*3–5*). Representative human smoking topography measurements (*6–11*) suggest that smokers of modern cigarettes smoke differently than the parameters specified by the FTC machine-smoking method (*12*).

Historically, the FTC's standardized machine-smoking protocol (*12*) has provided comparative information about the relative amounts of smoke constituents associated with cigarette use. In the United States, reports of tar, nicotine, and CO levels in mainstream cigarette smoke are still measured using the FTC protocol developed for higher tar and nicotine delivery cigarettes that were popular in the 1950–1960s. This protocol specifies that cigarettes are machine-smoked with a fixed puff volume (35 mL), puff duration (2 s), and puff interval (60 s). Mainstream smoke is drawn through a glass fiber filter pad, commonly called a Cambridge filter pad (CFP). Particulate smoke matter deposits onto the CFP while the vapor-phase portion passes through and accumulates in gastight bags. The mass difference of the CFP weighed before and after smoking measures total particulate matter (TPM). Nicotine and water extracted from the CFP are quantitatively measured, and tar is determined by subtracting the amounts of water and nicotine from the TPM value. In the FTC cigarette smoking protocol, CO is the only vapor-phase constituent measured.

Total exposure results from the contributions of both the particulate matter and the vapor-phase portions of cigarette smoke. However, most carcinogens, including the tobacco-specific nitrosoamines, polyaromatic hydrocarbons, heavy metals, and aromatic amines have been associated with cigarette smoke particulate matter (*13*). Several innovations in cigarette design helped achieve lower tar levels, as measured with the FTC protocol, including addition of a filtered tip, use of expanded tobacco, and inclusion of filter ventilation holes (*13*). Lower tar, as measured on a smoking machine, does not necessarily result in a comparable lowering of harmful chemicals delivered to a smoker. Djordjevic et al. (*14*) analyzed human smoking characteristics using a pressure transducer system to record data on the puff profiles during active smoking for medium- and light-delivery cigarettes. On average, human smokers took 48.6- or 44.1-mL puffs for medium- and light-delivery cigarettes with reduced puff intervals (21.3 and 18.5 s, respectively). When these smoking parameters were transferred to a smoking machine, the dosage of smoke constituents were dramatically higher than the levels measured using the FTC protocol.

The analysis of smoking topography data from numerous other studies (*6–11*) suggest that an average puff interval of 34 s and puff volumes of 43 mL are more typical for human smokers (*8*). The tobacco industry continues to use the FTC tar and nicotine values to arbitrarily classify and market cigarettes as either full-flavor (high delivery: >10 mg of tar), lights (medium delivery: ~5–10 mg of tar), or ultra-lights (low delivery: <5 mg of tar). Various researchers and public health advocates (*15–20*) have implied that all cigarettes are, in effect, designed with sufficient "elasticity" to give the individual smoker the means to satisfy his or her particular craving for nicotine despite the machine-generated lowered tar and nicotine levels of the light and ultra-light cigarette products.

To better estimate an individual's dosage to smoke constituents, we developed a quantitative method for the analysis of the solanesol content in the cellulose acetate portion of cigarette butt filters. Solanesol is a high molecular weight, nonvolatile, trisesquiterpenoid alcohol found as both the free alcohol and the leaf-bound solanesyl ester in leaf tobacco (2–3 wt %) and commercial tobacco products (*21–23*). Many

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reports on monitoring ETS have suggested that solanesol may be a potential marker for the cigarette smoke particulate matter dispersed in the environment (*24, 25*). The physical properties of solanesol that make it a useful ETS marker also can be applied to the analysis of mainstream cigarette smoke. In this study, we examine the applicability of measuring the solanesol content deposited in the cigarette butt filter after smoking to provide an accurate and noninvasive means to estimate the total amount of mainstream smoke, in terms of nicotine and tar, drawn through an individual cigarette.

Experimental Section

Sample Collection and Storage. Selected brands of commercial cigarettes were purchased at various retail outlets in the metropolitan Atlanta area. The unopened packs were assigned an identification number and logged into a database developed using Paradox (Borland Inprise, Scotts Valley, CA). The cigarettes were sealed using plastic bags in their original packaging and stored at −70 °C until tested.

Materials and Reagent Preparation. Solanesol was purchased from Sigma Sigma Chemical Co. (St Louis, MO). Ethyl triacontanoate (ET) was purchased from Aldrich Chemical Co. (Milwaukee, WI). Methanol, methylene chloride, hexane, toluene, and ethyl acetate were purchased from Burdick & Jackson Inc. (Muskegon, MI). Potassium hydroxide was purchased from Mallinckrodt Specialty Chemicals (Paris, KY). Organic pure water was prepared in house using a Solution 2000 water purification system (Solution Consultants Inc., Jasper, GA). All chemicals and solvents were checked for purity and used without additional purification. The 1 M KOH was prepared by dissolving 56 g of potassium hydroxide in a 1-L solution of 100 mL of organic pure water and 900 mL of methanol.

Standard Preparation and Characterization. Solanesol stock solution (1 μg/μL) was prepared by dissolving 20 mg of solanesol in 20 mL of toluene/ethyl acetate (9:1) solution. Likewise, an ET stock solution (10 μg/μL) was prepared by dissolving 200 mg of ET 20 mL toluene/ethyl acetate (9:1) solution for use as an internal standard. Dilutions were made to create a set of 11 final standard solutions encompassing a solanesol concentration range of 0.3−25 μg/mL. ET was added to each stock solution such that the final concentration of the internal standard was 50 μg/mL. A 5-μL aliquot of each standard solution was injected on column to obtain relative response factors. The total amount of solanesol injected on column to obtain a calibration curve ranged from 1.5 to 125 ng. It was possible to use diluted or more concentrated standards and maintain reasonable signal-to-noise values with good linearity; however, this was not necessary because the measured amounts spanned this range.

Sample Preparation. For sample preparation, the cigarette packs were removed from the freezer. Individual cigarettes removed from their packs were placed in labeled racks and conditioned for 24 h in a constant environmental chamber (22 °C, 60% relative humidity). The percent ventilation of each cigarette was determined using a QTM 5 (Filtrona, Milton Keynes, U.K.) pressure drop/ventilation measuring apparatus. After measuring ventilation, the cigarettes were smoked under standard conditions. Cigarettes were smoked using either a Filtrona SM342 (Filtrona Instruments & Automation Ltd., Milton Keynes, U.K.) or a Cerulean ASM500 (Cerulean, Milton Keynes, U.K.). The smoking machines were calibrated to take one puff of 2-s duration and 35-mL volume every 60 s and to maintain an average airflow velocity over the cigarette of 200 ± 30 mm/s unless otherwise noted. The standard puff volume (35.0 ± 0.1 mL) was measured using a 50-mL soap film glass buret (Filtrona Instruments Corp., Richmond, VA). Airflow velocity (200 ± 50 mm/s at the individual ports) was measured using a Filtrona VMD 100 velocity measurement digitizer (Filtrona Instruments & Automation Ltd., Milton Keynes, U.K.) connected to an Schiltknecht ThermoAir2 thermoelectric anemometer equipped with an omnidirectional probe (Schiltknecht Messtechnick AC, Gossau, Switzerland).

After the cigarettes were smoked, the filters from the cigarette butts were prepared for analysis. The outer paper wrapper was removed, and the filters were manually shredded using forceps to expose more surface area for extraction of solanesol. The filters were weighed and placed in 20-mL Teflon-lined screw-cap Qorpak vials. Quality control (QC) samples, consisting of clean cigarette filter material, usually referred to as "tow", spiked with 10 μL of a 1 μg/μL solanesol stock solution, were processed in tandem with the analytical samples. To each vial, 3 mL of 1 M KOH was added, and the vial was capped. The filter/base mixture was allowed to incubate in a heater block at 80 °C for 1 h. After the samples cooled to room temperature, aliquots of 6 mL of hexane and 60 μL of the internal standard stock solution were added to the vials. Sample vials were capped and rotated at 60 rpm for 30 min on a Glas Col Rotator (Terra Haute, IN). After removal from the rotator, the vials sat for several minutes to achieve layer separation before removal of 1 mL of the hexane extract. The extract was then diluted 1:1 with hexane to achieve a concentration of the internal standard at 50 μg/mL. The diluted extract was vortexed, and a 200-μL aliquot was pipetted into an autosampler vial for high-performance liquid chromatography (HPLC) tandem mass spectrometry (MS/MS) analysis. An injection of 5 μL of sample provided an adequate signal-to-noise ratio even though this represents consumption of only 1 part in 2400 (5 μL of a 6-mL extract diluted 1:1) of the total solanesol extracted. The dilution factor is taken into account during the calculation of the amount of solanesol in each sample. Although the solanesol is present in the filters at microgram quantities, only nanogram levels were injected on column.

Instrumentation and Analysis. The samples were analyzed using normal-phase HPLC MS/MS with atmospheric pressure chemical ionization (APCI) ionization on a Perkin-Elmer SCIEX 3000 mass spectrometer (Applied Biosystems, Forest City, CA) coupled with a Hewlett-Packard 1100 liquid chromatograph (Palo Alto, CA). Separation was achieved using a 15 cm × 4.6 mm Chromega Amino/Cyano column with 60-Å pores and 5-μm particle diameter (Bodman, Aston, PA). The HPLC was operated in an isocratic mode using a solvent consisting of 66% dichloromethane, 33.5% hexane, and 0.5% methanol with a flow rate of 500 μL/min. Complete chromatograms were obtained with a 7-min run time.

The HPLC effluent was ionized in the APCI source using a nitrogen nebulizing gas in the positive-ion mode. The APCI vaporizer and capillary temperatures were 300 and 200 °C, respectively. The mass spectrometric detector, at unit mass resolution, was scanned at 0.75 s/amu. The instrument was operated to select the ET and solanesol precursor ions at m/z 482 and m/z 632, $[M + H]^+$, respectively. Following collision-induced dissociation, the remaining precursor ion abundance and corresponding product ions (m/z 454 and m/z 614) were monitored. Relative response factors for solanesol were determined by calculating the ratio of the reconstructed ion chromatogram peak areas of the solanesol and ET product ions. The calculated peak areas were saved as a file and transferred from the instrument to a PC-based compatible workstation. The peak area data from all samples, daily calibration curves, QC checks, and blanks were imported into a database environment, R:BASE (Microrim, Redmond, WA), for subsequent processing and analysis. Linear regression of the calibration data was done using custom programs developed with SAS statistical software (SAS Institute, Cary, NC). Final reported values were calculated within R:BASE and included corrections for sample dilutions and purity of

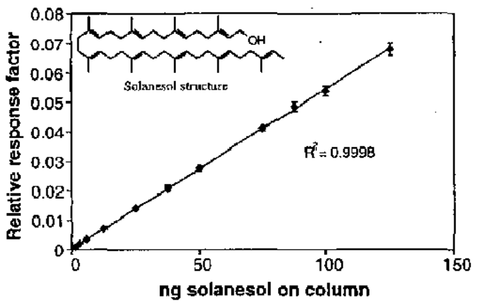

FIGURE 1. Typical calibration curve from the analysis of solanesol deposited in the cellulose acetate filter of a cigarette butt. Error bars represent 95% confidence intervals.

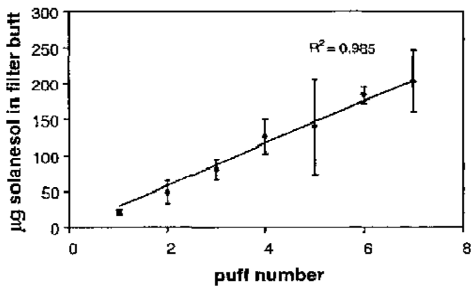

FIGURE 2. Relation between the amount of solanesol deposited in the cigarette filter and the number of puffs. Error bars represent 95% confidence intervals.

TABLE 1. Variation in Nicotine, Tar, and Solanesol Deposited in the Cigarette Filter Butt with Different Amounts of Filter Vent Hole Blockage

% filter vent blockage	nicotine (mg/cigarette)	tar (mg/cigarette)	solanesol (µg/cigarette)
0	0.16 ± 0.02[a]	2.02 ± 0.36	52.6 ± 10.6
50	0.62 ± 0.05	6.63 ± 0.95	157.3 ± 11.1
100	1.37 ± 0.08	19.59 ± 0.40	248.9 ± 23.2

[a] Standard deviation.

the standards. All other statistical analyses, including QC evaluations, were performed using SAS.

Results

Calibration Results. Daily calibration of the LC/MS/MS instrumentation with an 11-point curve corresponding to a range of 1.5−125 µg of solanesol yielded calibration curves with excellent linearity and reproducibility (Figure 1). Using the SAS software, we obtained calibration constants for the least-squares fit. Correlation coefficients from the standard curves typically ranged from 0.998 to 0.999. The *y*-intercepts, calculated from the least-squares fit of the data, did not significantly differ from zero. Standard deviations (SDs) from replicate analysis of the calibration curves were used to calculate the limit of detection (LOD), equal to $3\times S_0$ (*26*), the standard deviation at zero concentration (Table 1). The LOD for solanesol was calculated as 1.5 ng injected on column.

Reproducibility and Quality Controls. Multiple samples of 20-mm lengths of cellulose acetate filter material were spiked with a known amount of solanesol and analyzed to establish QC criteria. In the United States, cigarette filters are typically derived from long rods of cellulose acetate fibers of specified length, diameter, and efficiency. The 95% and 99% confidence intervals were calculated for solanesol content in the spiked filter material. Acceptance criteria followed the QC protocol suggested by Taylor (*26*).

Sample Recovery. The analytical recovery for this method was determined by five replicate measurements of 5, 10, and 20 µg of solanesol spiked on 20-mm sections of cellulose acetate tow. The samples were processed under conditions identical to the cigarette butt filters. The amount of solanesol in the spiked samples was quantitatively determined and compared with the amount added to determine the percent recovery. The average percent recovery was 99.85%, with a relative SD of 5.35%. No statistically significant differences in the calculated recovery were observed for the three different spike amounts.

Method Validity. Several experiments were devised to determine whether the amount of solanesol, deposited in the filter of a cigarette butt during smoking, was proportional to the amount of smoke that had passed through the filter. A typical modern king-size cigarette yields 6−10 puffs when smoked according to FTC puffing conditions. Therefore, we varied the number of puffs to examine whether the amount of solanesol deposited in the cigarette filters was related to the amount of smoke passing through the cigarette filter. A series of Marlboro Light cigarettes were smoked, in triplicate, with the puff number per cigarette sequentially increased from one to seven. The filters from the resulting 21 cigarette butts were quantitatively analyzed for total solanesol. The solanesol content in these filters increased with the puff count, and good linearity was observed with a correlation coefficient of 0.985 when the least-squares regression analysis was forced through the origin (Figure 2). The slope determined from the least-squares linear regression analysis of these data indicated that the solanesol content in the filters of the Marlboro Light cigarettes increased, on average, by 29.25 µg/puff. The per puff deposition rate of solanesol in other cigarette brands may vary depending on key physical characteristics such as the packing density of the tobacco, the porosity of the paper wrapper, and the amount of filter ventilation. Further studies are required to determine the consistency between brands and from lot to lot of the same brand.

Cigarette delivery is obviously influenced by puff volume. The larger the puff volume, the more smoke is drawn through the cigarette and into the smoker. Unlike the fixed 35-mL puff volume used by the FTC method, numerous researchers (*6−11, 14*) have reported that modern cigarettes are usually smoked with larger puff volumes. A series of Carlton 100 cigarettes were smoked in triplicate using a 60-s puff interval, 2-s puff duration, with puff volumes of 35, 45, 55, 65, and 75 mL to assess the influence of puff volume on smoke delivery and solanesol deposited in the filter. Not surprisingly, as the puff volume increased, the amount of nicotine and tar in the mainstream smoke and the amount of solanesol deposited in the filter also increased. Figure 3 shows the levels of mainstream smoke tar and nicotine plotted against the solanesol filter content measured with puff volumes of 35, 45, 55, 65, and 75 mL. Least-squares linear regression analysis of these data demonstrated a good correlation between the mainstream smoke deliveries of tar and nicotine with respect to the filter solanesol measured at the different puff volumes. Correlation coefficients of 0.940 for tar and 0.978 for nicotine were observed for these data.

The delivery of a cigarette also can be influenced by filter ventilation. Blockage or obstruction of the filter ventilation holes provides an additional means to vary the amount of smoke passing through the filter. Tests were conducted to determine how the solanesol content in the filters from cigarette butts varies as the amount of filter ventilation decreased. A series of Carlton 100 cigarettes, where the filter ventilation holes had been unobstructed (0% blocked), partially blocked (50% blocked), or completely blocked (100%

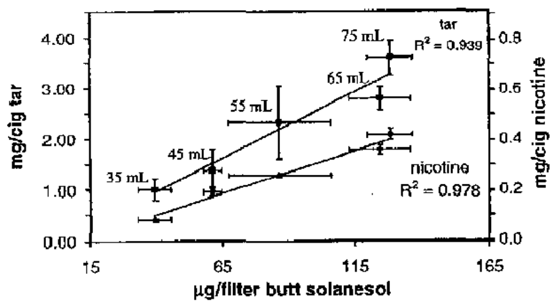

FIGURE 3. Relation between the mainstream tar and nicotine deliveries and solanesol deposited in the filter obtained at 35-, 45-, 55-, 65-, and 75-mL puff volumes. Error bars represent 95% confidence intervals.

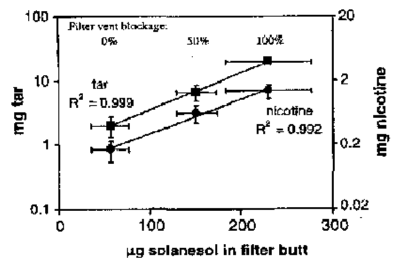

FIGURE 4. Relation between the mainstream tar and nicotine deliveries and solanesol deposited in the filter 0%, 50%, and 100% filter ventilation hole blockage. Error bars represent 95% confidence intervals.

blocked) using small strips of cellophane tape, were machine smoked. An increase in the tar (977%) and nicotine (822%) was observed on the CFP when comparing the smoke particulate phase collected when the vent holes were completely blocked versus when they were not blocked. The solanesol content increased by 473% in the completely blocked cigarette filters relative to unblocked vent holes. Table 1 shows the measured mainstream smoke deliveries of tar and nicotine and the amount of solanesol deposited in the filters of cigarette butts measured with increasing amounts of filter vent hole blockage. Linear regression correlation coefficients (R^2) of 0.899 and 0.963 were obtained from an analysis of the measured tar and nicotine plotted as a function of the amount of solanesol deposited in the filters. However, linearity improved when the logarithm of tar and nicotine was plotted against the filter solanesol content. A semilog plot of these data (Figure 4) provides improved values for the correlation coefficients of 0.999 for tar and 0.992 for nicotine.

For the measurement of solanesol levels in cigarette butt filters to provide a viable means for estimating mainstream smoke deliveries and usage patterns, these levels must be stable during storage at room temperature. To test the stability of these materials, we examined the concentrations of solanesol in the cigarette butt filters from a series of identical machine-smoked cigarettes as a function of storage time. A series of 15 Marlboro Light cigarettes were smoked, and the filters were removed from the butts and stored in resealable plastic bags at room temperature. Immediately after the cigarettes were smoked, the solanesol content in three of the filters was determined. The remaining filters were analyzed in triplicate during the following 4 weeks. The solanesol content measured in these filters versus storage time is shown in Figure 5. Losses in solanesol during the 4-week period appeared to be minimal. Within the range of experimental error, no statistically significant difference existed in the solanesol content. However, a general downward trend appears to exist. The slope of a least-squares linear regression

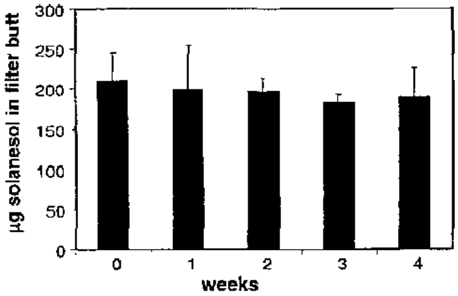

FIGURE 5. Change in solanesol in cigarette filter butts stored in plastic bags at room temperature. Error bars represent 95% confidence intervals.

analysis suggests that the rate of solanesol loss rate is no more than 0.4% per day.

Discussion

The measurement of solanesol content deposited in the filter of a cigarette butt meets the criteria for a good smoke marker. The amount of deposited solanesol is a function of the mainstream smoke delivery of nicotine and tar. Its measurement provides a noninvasive means to access mainstream cigarette smoke exposure, allows for both individual data on a per cigarette basis and cumulative exposure estimates, and does not require real-time monitoring. The data also suggest that solanesol analysis is applicable for a relatively long time after smoking has occurred. The solanesol in cigarette butt filter method appears to provide adequate sensitivity and reproducibility to provide dosage estimates. This method utilizes automated sample injection and a 7-min LC/MS/MS run time to achieve rapid and accurate solanesol quantitation with high sample throughput.

The applicability of this technique has several potential limitations. This procedure has only been tested with cigarettes containing a cellulose acetate filter. However, most cigarettes smoked in the United States have this type of filter. Other novel filter types such as paper, combination paper and cellulose acetate, or graphite-impregnated cellulose acetate may be applicable to this type of analysis, but this has not yet been confirmed. Also, if under extreme smoking conditions the filter became saturated and breakthrough occurred, the solanesol analysis would provide only a lower estimate of smoke dosage. This may be the effect that is starting to be observed for the filter vent hole blockage data shown in Table 1. Linear behavior was observed for filter solanesol as a function of puff number and as a function of puff volume for both a light and ultra-light delivery cigarette. As the tar deliveries increased above the 10–15-mg range, noticeable deviation from linearity was observed. This likely resulted from the elevated levels of cigarette smoke constituents, including solanesol, associated with such high tar levels and the filter could be approaching saturation. Our findings suggest that, even at these elevated smoke delivery levels, we can obtain good linearity using a semilog plot up to and possibly beyond a 20-mg tar delivery. We believe this should be adequate to obtain reasonably accurate smoke intake estimates on most modern domestically produced filtered cigarettes. Other factors could also affect the relation between mainstream smoke deliveries and filter solanesol. Various components in the tobacco filler or blend could alter the ratio of solanesol to nicotine or tar. Differences in the filter type, efficiency, or retention could alter solanesol levels in the butts. Either of these cases would require calibration for each cigarette brand. Further studies are planned to evaluate extent to which this concern increases the necessity of individual brand calibration.

Although smoking machine derived tar levels in cigarettes have dramatically dropped since the 1950s, tobacco use remains the leading cause of preventable death in the United Stares (*27*). An unintended consequence of lowing tar delivery in cigarettes may have resulted in an increase in certain type of lung cancers (*28*). A more rigorous machine smoking protocol to better mimic contemporary human smoking behavior should provide more realistic estimates of smoke exposure. However, such an approach only provides aggregate data and would not be able to examine individual smoking characteristics or mimic the variation in each person's smoking parameters that vary throughout the day. A much more informative but perhaps less practical approach would be the use of an invasive technique to collect biological samples from a representative population of human smokers to assess smoke dosage. Such measurements should provide the best possible smoke constituent dosage estimates from analysis of biological samples for key smoke markers such as nicotine; its major metabolite, cotinine; or other smoke biomarkers in blood or serum. Noninvasive collection of urine or saliva would provide a means to measure smoke intake levels, but have similar problems associated with collecting and storing other biological samples. The inherent expense and difficulty in processing large numbers of biological samples from individual smokers limits the utility of such approaches.

Measurement of biomarkers, even when made under carefully controlled settings, is not likely to reflect subtle differences in cigarette-to-cigarette usage patterns. For example, epidemiologic findings suggest that the time after waking to the first cigarette of the day is a significant predictor of addiction (*29*) and thus may be smoked differently to those consumed later in the day. In addition, differences in smoking patterns may be influenced by external factors. Consumption patterns in a relaxed recreational or leisure setting may vary significantly from those in a stressful work environment or during a break in a smoke-free workplace. Ideally, a quick and simple noninvasive method for monitoring smoke uptake levels over the course of 1 day or for longer would provide useful information about individual smoking practices. Data obtained on numerous smokers, when taken in aggregate, should provide a basis for more realistic estimates of smoke dosage. This information could help establish improved smoking protocols to access the exposure potential of diverse smoker populations using various cigarette products that differ in smoke delivery potentials.

To be a useful representative surrogate exposure measurement for smoke intake, a chemical marker should be easily measured, related to the amount of exposure, and stable. Prior noninvasive methods for assessing smoke exposure had potential shortcomings in terms of time constraints, required training of personnel, or difficulty in implementation. Standardized machine smoking under FTC protocol seriously underestimates the smoke deliveries for light and ultra-light cigarette products. Questionnaires to ascertain an individual's smoking pattern depend on smoker recollection and are subject to bias. Smoking topography measurements can provide valuable insights in smoking behavior; however, the equipment used in this method perturbs the normal smoking process and precludes some compensation because the transducer effectively prevents deliberate or inadvertent filter vent hole blockage.

Numerous studies have used invasive means to collect chemical biomarkers such as nicotine (*30*), cotinine (*31*), or hemoglobin adducts (*32*) in samples of blood, urine, or saliva. With the exception of measuring expired CO levels (*33*), these techniques often require a time-consuming sample collection protocol yielding samples that require refrigeration or other types of special handling to ensure analyte integrity. Therefore, in many instances, constraints often severely limit the utility of invasive procedures and restrict their use to relatively small sample populations. Other techniques, such as expired CO and heart rate monitoring measurements, also provide for relatively noninvasive data collection but are applicable during or shortly after smoking and require trained personnel and equipment during or immediately after smoking.

Hopkins et al. (*34*) have measured a number of physiologic and biological markers of cigarette use, including plasma nicotine and cotinine levels, and attempted to correlate such findings with the nicotine content in the filters of cigarette butts after smoking. The nicotine content in the cigarette filter was not well-correlated with any of the biological smoking markers. The authors concluded that nicotine in the cigarette butt filter would not provide an accurate estimate of smoke uptake. Such findings may not be surprising because nicotine is known to be a relatively volatile compound. Experiments by Sensabaugh and Cundiff (*35*) showed that cigarette smoke is more alkaline than tobacco, which would facilitate the equilibrium shift from protonated to the volatile free-base form of nicotine. Solanesol because of its high molecular weight, long chainlike structure, and low vapor pressure would not be subject to such evaporative losses.

Solanesol has been used as a marker for ETS in numerous studies. Nelson et al. (*36, 37*) found that the fraction of solanesol present in ETS typically varies from 1.5% to 4%, depending on the cigarette blend. Compared with other commonly used chemical markers, including CO (*38*), nicotine (*39, 40*), scopoletin (*41*), and mysomine (*42*), solanesol is considered a good marker for particulate matter associated with ETS (*24, 25*) with a relatively slow decay rate. Solanesol in the environment has been speculated to undergo UV radiative degradation (*25*). However, the solanesol trapped inside the cigarette filter is only minimally exposed to light at the open ends. Therefore, unlike the decay observed for solanesol associated with ETS, solanesol in filters is stable for many days. In fact, our results suggest that the samples can be stored at room temperature and analyzed weeks later.

The concept of cigarette equivalents (CE) formulated by Hoegg (*43*) and further refined by Ogden and Martin (*44*) has been used to estimate ETS exposure. Both ETS and cigarette smoke contain numerous chemical constituents, and one chemical marker is unlikely to reflect the behavior as a whole. In particular, whether one chemical marker can serve as a good surrogate measurement of the toxic or carcinogenic constituents in a dynamic and time-dependent concentration situation as found in ETS is not known. This and other limitations has led to the conclusion by some that CE is a less-than-desirable means of estimating potential risk associated with ETS exposure (*45*). However, we found that the filter solanesol concentrations related well to the levels of tar and nicotine measured in mainstream smoke deliveries from individual cigarette brands.

The amount of tobacco consumed, filter ventilation level, puff frequency, and puff volume greatly influence the delivery of tar and nicotine to the smoker. Under the FTC protocol, the smoking parameters do not vary. However, variation in human smoking patterns greatly alters tar and nicotine delivery. Herein lies the greatest challenge in using standardized smoking protocols to estimate human exposure. Two individuals can smoke the same brand of cigarette and through various compensation mechanisms (i.e., larger puff volumes, filter vent blocking, shorter puff intervals) can, and probably do, achieve different levels of exposure. Thus, the filter solanesol measurement we developed provides a means to estimate exposure to mainstream cigarette smoke from cigarettes that contain a cellulose acetate filter regardless of the number of puffs, the inhaled puff volume, or the level of filter ventilation hole obstruction. Future studies are planned to examine how the solanesol content deposited in the filter of cigarette butts varies over a range of cigarette products

and whether solanesol content will serve as a useful marker for other constituents in mainstream smoke.

The measured level of solanesol in the filter provides a useful marker for estimating smoke uptake regardless of how the cigarette was smoked. Such information helps make useful comparisons of individualized smoking experiences in different situations. Accumulation and examination of such information from a representative population of smokers should provide insight into current usage patterns, help evaluate the range of individual smoking behavior, and ultimately help develop more realistic machine-smoking protocols. One of the most important aspects of this approach is that it provides a noninvasive means for estimating individualized cumulative smoke dosage and allows the time-dependent exposure or situation-dependent changes in smoking patterns without involving equipment or personnel during the smoking event. The smoke profile and exposure estimates could be determined days or weeks after collection of the cigarette butts. Because the method is sensitive, accurate, and capable of high throughput, sampling of large populations is feasible for determining the differences in cigarette smoking patterns on the basis of numerous important variables such as brand preference, age, race, or sex.

Acknowledgments

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